SCIENCE

Bradykinin Inhibition by Butylated Hydroxyanisole

Abstract. *Concentrations of butylated hydroxyanisole as low as 8×10^{-9} mole per liter can inhibit detectably the contraction of smooth muscle elicited by bradykinin. The mechanism of the inhibitory effect of this food grade anti-oxidant is apparently complex, and the effect is only partially reversible.*

Bradykinin's contractive action on smooth muscle is inhibited by agents generally classed as tranquilizers (*1*). We now report similar inhibition of bradykinin activity by butylated hydroxyanisole (BHA), an antioxidant widely used for the preservation of foods containing unsaturated lipids.

Assays of this inhibitory effect were made with a 2- to 3-cm section of the terminal segment of a guinea pig ileum suspended in a 5-ml capacity perfusion bath held at 37°C. Changes in length of the ileum were recorded with a standard kymograph. The gut was equilibrated against Tyrode's buffer containing 2 mg of atropine and 40 μg of pyribenzamine per liter by flowing the solution through the cell at a rate of 15 ml per minute for 1 hour before use.

After equilibration the flow of Tyrode's buffer through the cell was suspended, and a 0.2-ml portion of test solution containing pure bradykinin and the antioxidant were injected into the cell. Test solutions were made from stock solutions of butylated hydroxyanisole (Nutritional Biochemicals) dissolved in water and synthetic bradykinin (BRS 640, Sandoz) dissolved in Tyrode's buffer. After exposure for 30 seconds to a test solution, the mixture was flushed from the cell, and the entire procedure was repeated with another kinin mixture. The response of the gut to a solution containing only pure kinin was used throughout the analyses as a control. The height of the peak traced on the kymograph chart when the gut was in contact with the kinin-containing solution was taken as

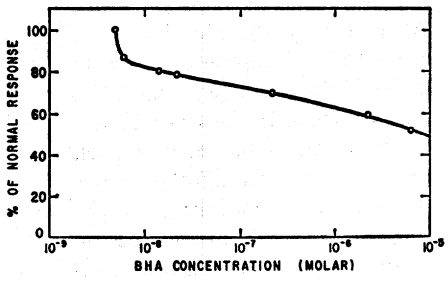

Fig. 1. Suppression of ileum response to test solutions of bradykinin containing increasing amounts of BHA. Gut was freed of reaction mixtures by flushing Tyrode's buffer through the cell at a rate of 15 ml/min for 10 minutes between each point determination. The raw data were corrected for irreversible decreases in gut sensitivity due to exposure to BHA.

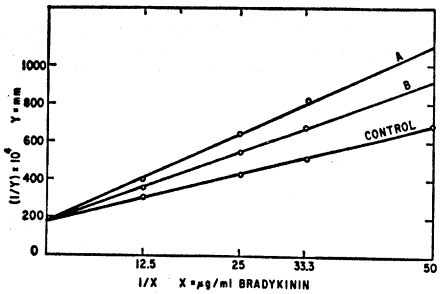

Fig. 2. Drug dose (x)–response (y) plot characterizing suppression of contractile response of smooth muscle (guinea pig ileum) by BHA. Control samples contain no antioxidant. Points on line A were obtained with 7.2×10^{-9} g of BHA per milliliter in test solution, and points on line B were obtained with 3.6×10^{-9} g of BHA per milliliter. All points represent data corrected for loss in gut sensitivity to bradykinin due to exposure to BHA.

being directly proportional to the amount of smooth muscle contraction elicited by bradykinin. Reduction in peak height by added antioxidant was taken as a measure of its inhibitory action.

With this procedure, it was found that the threshold concentration at which BHA began to suppress the ileum's response to 0.04 μg of bradykinin per milliliter in the reaction bath was $8 \times 10^{-9}M$. Increasing concentrations of antioxidant depressed gut response (Fig. 1).

Supposedly the mechanism of this inhibition can be ascertained from a standard study of the relation of drug to dose response as described by Roche e Silva (*2*). Here the reciprocals of effects ($1/Y$), where Y is peak height in millimeters, as measured on the kymograph chart are plotted against the reciprocals of the doses ($1/X$), where X is equal to the concentration of bradykinin in the reaction chamber. Our data in this form are presented in Fig. 2. From the relatively straight line plots and the common intercept shown in Fig. 2 it can be inferred (*2*) that BHA acts as a competitive inhibitor of bradykinin. Likewise, from the second plot, the relative inhibitor strength of BHA can be derived (*2*) as its inhibitory potency (pK_1). The value ascertained by this procedure, $pK_1 = 7.9$ g/ml, indicates that BHA is a relatively strong inhibitor.

The validity of this relation may be questioned since the inhibitory action of BHA is not completely reversible. After four 30-second exposures to solutions containing $1 \times 10^{-6}M$ BHA stock solution, only 92 percent of normal bradykinin response could be recovered by flushing with buffer for 15 minutes. The reason for this loss in sensitivity has not been established. It probably involves very strong or irreversible binding of the antioxidant on some bradykinin receptor sites since the smooth muscle contracts normally in response to 0.40 μg of histamine per milliliter in the presence of $10^{-5}M$ concentrations of BHA in the reaction bath. Regardless, our data demonstrate that relatively low concentrations of BHA can inhibit the contractile action of bradykinin on smooth muscle. The higher BHA concentrations used in these experiments approach the concentrations of BHA permitted in foods under present Food and Drug Administration regulations. The significance of this finding in regard to the widespread use of this antioxidant in food manufacture remains to be established.

The possibility that an impurity in the commercial BHA sample used in our study could have given rise to the described results has been considered. Standard gas-liquid chromatographic analysis of the antioxidant used showed it to be essentially 7 percent 2-BHA and 93 percent 3-BHA. Only two volatile contaminants in trace quantities could be found on the chromatogram. A sample of BHA further purified by sublimation also has the same inhibitory power as the commercial sample. Furthermore, we have some data that indicate other phenolic antioxidants similarly inhibit the response of smooth muscle to bradykinin.

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References

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2. M. Roche e Silva, *Arch. Int. Pharmacodyn.* **118**, 74 (1959).

20 November 1969; revised 12 January 1970